ASBMT Best Abstract Awards for Basic Science

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PLEIOTROPHIN SIGNALING IS NECESSARY FOR HEMATOPOIETIC STEM CELL SELF RENEWAL AND IS REGULATED BY THE BONE MARROW MICROENVIRONMENT

Himburg, H.A.[1], Daher, P.[1], Russell, L.[1], Doan, P.[1], Quarmyne, M.[1], Meadows, S.[1], Herradon, G.[2], Chao, N.J.[1], Chute, J.P.[1] [1] Duke University Medical Center, Durham, NC; [2] University San Pablo CEU, Madrid, Spain

We recently demonstrated that treatment of murine and human HSCs with the heparin binding growth factor, pleiotrophin (PTN), was sufficient to induce self-renewal of murine and human HSCs in culture (Himburg, Nat Med, 2010). In order to determine if PTN signaling is necessary for HSC self renewal and normal hematopoiesis *in vivo*, we examined mice bearing a constitutive deletion of PTN (PTN −/− mice) (Jackson Laboratory). PTN −/− mice demonstrated no significant differences in total bone marrow (BM) cells or BM colony forming cells (CFCs), but had significantly decreased bone marrow CD34(-)c-kit(+)sca-1(+)lin(-) (34-KSL) cells (0.007% vs. 0.02%, p = 0.03) and 2–fold decreased CFU-S12 content compared to control mice that retained PTN (PTN +/+ mice)(p = 0.003). Importantly, PTN −/− mice demonstrated an 11-fold decrease in long-term repopulating HSC content compared to PTN +/+ mice as measured by competitive repopulating unit (CRU) assay (12 week CRU frequency: 1 in 6 cells vs. 1 in 66 cells). To determine whether the effect of PTN deletion on HSC content was cell autonomous, we generated chimeric mice by transplantation of CD45.1 congenic BM cells into lethally irradiated CD45.2 PTN−/− or PTN+/+ mice. After 8 weeks of transplantation, the number of BM 34-KSLs and BM CFU-S content were significantly decreased in the recipient PTN−/− compared to the PTN+/+ mice (p = 0.04). These data demonstrated that the bone marrow microenvironment is necessary for PTN signaling with HSCs. By quantitative real time PCR, we found that both BM osteoblasts and endothelial cells in wild type mice express PTN. In addition, treatment of non contact cultures of BM KSL cells and primary BM ECs with anti-PTN blocked the capacity of BM ECs to support the expansion of CFC and CFU-S in vitro compared to BM EC cultures treated with isotype antibody (p = 0.01 and p < 0.0001, respectively). These data demonstrate that PTN is produced by BM ECs in the HSC niche and PTN signaling is necessary for maintenance of the BM HSC pool *in vivo*.

2

MANIPULATION OF THE BIOENERGETICS OF ALLOREACTIVE T CELLS LEADS TO THEIR SELECTIVE APOPTOSIS AND ARRESTS GRAFT VERSUS HOST DISEASE

Wahl, D.R.[1], Gatza, E.[1], Opipari, A.W.[1], Sundberg, T.B.[1], Reddy, P.[1], Liu, C.[2], Glick, G.D.[1], Ferrara, J.L.M.[1] [1] The University of Michigan, Ann Arbor, MI; [2] The University of Florida, Gainesville, FL

Proliferating cells fuel division by increasing ATP production through oxidative phosphorylation (OXPHOS) or glycolysis. Following bone marrow transplantation (BMT), both hematopoietic stem cells (HSCs) and alloreactive T cells rapidly proliferate to reconstitute the immune system or mediate graft versus host disease (GVHD), respectively. Proliferating HSCs and progenitor cells on d 8 after syngeneic BMT (B6→B6-Ly5.2) increased glycolysis 3-fold (p < 0.0001, Table 1) but did not increase OXPHOS. By contrast, alloreactive donor T cells in a B6→B6D2F1 model of GVHD more than doubled OXPHOS compared to unstimulated T cells (p < 0.0001, Table 1), which accounted for 80-90% of ATP produced. These results are surpising, as lymphocytes stimulated in vitro generate ATP primarily through glycolysis. Alloreactive donor T cells also proliferated twice as fast as proliferating bone marrow cells (p < 0.0001, Table 1). The more rapid proliferation and increased OXPHOS in alloreactive donor T cells was associated with increased oxidative stress, as indicated by increased reactive oxygen species (ROS), and a loss of the antioxidants pyruvate (98% decrease, p = 0.004) and glutathione (25% decrease, p = 0.04). Proliferating bone marrow cells did not exhibit this abnormal bioenergetic phenotype (Table 1). We exploited these redox abnormalities using Bz-423, a novel benzodiazepine that modulates the mitochondrial F_1F_o-ATPase and induces ROS-mediated apoptosis. One injection of Bz-423 increased ROS production 2-fold in alloreactive donor T cells (p < 0.001), and increased their apoptosis by 50% (p < 0.001). Bz-423 did not produce ROS or induce apoptosis in unstimualted T cells, B cells or proliferating bone marrow cells. Furthermore, 6 doses of Bz-423 significantly decreased mortality in several models of GVHD (B6→F1: 60% vs. 0%, d 21, p < 0.03; Balb/c→B6: 100% vs. 40%, d 60, p < 0.02; C3H.SW→B6: 71% vs. 25%, d 80, p < 0.02) and significantly reduced GVHD-associated pathology in the liver and intestine. Importantly, Bz-423 treatment did not impair immunologic reconstitution or donor engraftment. The beneficial effects of Bz-423 were due to its ability to induce apoptosis, as treatment with a pan-caspase inhibitor abrogated any survival benefit. We conclude that alloreactive donor T cells have an abnormal metabolic phenotype evidenced by increased OXPHOS and depleted antioxidants that can be exploited for selective immunotherapy of GVHD without inhibiting hematopoeitic reconstitution.

Table 1.

	OXPHOS (nMoles O_2/(min*10^6 cells))	Glycolysis (nMoles lactate/ (min*10^6 cells))	Pyruvate (pMoles/μg protein)	Proliferation Index
Unstimulated T	0.20 (0.03)	0.07 (0.02)	10.4 (3.0)	-
Alloreactive donor T	0.48 (0.05)*	0.53 (0.07)	0.2 (0.1)*	3.5 (0.04)*
Proliferating Bone Marrow	0.23 (0.05)	0.90 (0.11)*	8.5 (2.3)	1.7 (0.07)

*p<0.05

3

GENE THERAPY TARGETING SYNTHESIS OF COAGULATION FACTOR VIII IN PLATELETS REDUCES BLEEDING IN CANINE HEMOPHILIA A

Du, L.M.[1], Franck, H.W.G.[2], Merricks, E.P.[2], Nurden, P.[3], Jensen, E.S.[4], Haberichter, S.L.[1,5], Hawkins, T.B.[6], Jacobi, P.M.[1], Fang, J.[1], Nichols, T.C.[2], Koukouritaki, S.B.[1], Shi, Q.[1,5], Nurden, A.T.[3], Montogomery, R.R.[1,5], Cornetta, K.[6], Wilcox, D.A.[1,5] [1] Children's Research Institute, Children's Hospital of WI, Milwaukee, WI; [2] University of North Carolina, Chapel Hill, NC; [3] Hôpital Xavier Arnozan, Pessac, France; [4] Medical College of Wisconsin, Milwaukee, WI; [5] BloodCenter of Wisconsin, Milwaukee, WI; [6] Indiana University School of Medicine, Indianapolis, IN

To develop a clinically relevant strategy for gene therapy of inherited bleeding disorders, we investigated if Factor VIII (FVIII) could be stored in platelets derived from lentivirus-transduced hematopoietic stem cells of dogs with Hemophilia A. This should allow the release of FVIII from activated platelets as a hemostatic response to a severe bleeding episode. cG-CSF/cSCF mobilized peripheral blood stem cells (PBSC) were immuno-selected for CD34 from an apheresis product, transduced with a lentivirus encoding the platelet-specific integrin αIIb gene promoter driving expression of human FVIII. The PBSC (2 x 10^6/kg) were autologously transplanted (Tx) into animals preconditioned with Bulsulfan (5-10 mg/kg i.v.). Three Tx recipients underwent periodic testing for expression of the FVIII transgene as well as immune tolerance and phenotypic correction of Hem A. LAM PCR localized the lentivirus in genomic DNA isolated from leukocytes of each dog. Immunofluorescence confocal microscopy detected FVIII in a subset of platelets. Immune electron microscopy revealed that FVIII was stored in the platelet α-granules. Chromogenic analysis of the platelets demonstrated that FVIII was

present in its biologically active form (FVIII:C) at approximately 5 mU/ml/10^8 platelets from 20 wks through 2 yrs after PBSC Tx. In contrast, FVIII:C was not detected within the plasma of these animals. This result coupled with the use immunomodulation drugs may help to explain why the dogs remained tolerant of human FVIII as indicated by our inability to detect inhibitory antibodies to FVIII. Following successful gene transfer and PBSC engraftment, all animals showed signs of clinical improvement of Hem A: the 1st Tx recipient had 1 bleed/yr for 2 yrs (vs expected 5/yr), the 2nd dog has not bled for 20 months following PBSC Tx and the 3rd animal had no bleeding for 10 months post Tx. In addition, the 1st Tx dog showed recovery from prolonged history of chronic gastrointestinal bleeding. All 3 animals displayed improved whole blood clotting times. This data is consistent with our previous results demonstrating synthesis, trafficking, storage and release of FVIII from α-granules of human megakaryoyctes *in vitro* and platelets of the murine model for Hem A. The outcome demonstrates a feasible strategy for controlling severe bleeding episodes in patients with Hem A by providing a locally inducible secretory pool of FVIII in platelets derived from an autologous Tx of FVIII-transduced PBSC.